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DRUG EVALUATION IN THE PLASMODIUM FALCIPARUM-AOTUS MODEL

ANNUAL REPORT

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June 14, 1990

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick
Frederick, Maryland 21701-5012

Contract No. DAMD17-87-C-7163

Gorgas Memorial Institute of Tropical &
Preventive Medicine, Inc.
9650 Rockville Pike
Bethesda, MD 20814

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Gorgas Memorial Institute of Tropical and Preventive Medicine 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 9650 Rockville Pike Bethesda, MD 20814	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Contract No. DAMD17-87-C-7163
8c. ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62787A; PROJECT NO. 3M1 62787A870; TASK NO. AJ; WORK UNIT ACCESSION NO. WUDA312790

11. TITLE (Include Security Classification)
Drug Evaluation in the Plasmodium falciparum - Aotus Model

12. PERSONAL AUTHOR(S)
Richard N. Rossan

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 5/15/89 TO 5/14/90	1990 June 14	54

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Plasmodium falciparum; Aotus l. lemurinus; artemisinin derivatives; in vivo reversal of chloroquine resistance
06	13		
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Blood-induced infections of the multidrug resistant Vietnam Smith/RE strain of Plasmodium falciparum in Aotus lemurinus lemurinus were used for antimalarial drug evaluation studies. Studies with four derivatives of artemisinin, the active principal of the Chinese herb qinghao, were completed. The two water-soluble derivatives, WR 256283 (artesunate) and WR 255663 (artelinate), administered at doses of 16.0, 32.0, and 64.0 mg/kg (i.m., q.12hx3), cleared parasitemias, but did not cure infections. The two oil-soluble derivatives, WR 255131 (arteether) and WR 254986 (artemether), were administered (i.m.,q.12x3) at doses ranging from 0.25 to 64.0 mg/kg in primary treatments and retreatment In a total of 58 treatments, arteether cured 36 (62%) infections. Artemether cured 35 infections out of 49 (71%) treatments. The calculated ED_{50} for arteether is 10.0 mg/kg and for artemether is 4.0 mg/kg.

In a continuation of experiments to reverse chloroquine-resistance in vivo, two neuroleptic phenothiazines, chlorpromazine (WR 2173) and prochlor-

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mary Frances Bostian	(301)663-7325	SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete*

SECURITY CLASSIFICATION OF THIS PAGE

19. perazine (WR 6379), were each administered with chloroquine, once daily for 7 days. Primary treatment with chlorpromazine (10.0 mg/kg) plus chloroquine (20.0 mg/kg) cleared parasitemia in each of two Aotus and cured the infection in one animal. Primary treatment with chlorpromazine (20.0 mg/kg) plus chloroquine (20.0 mg/kg) cured infection in 2 of 2 Aotus. Prochlorperazine (20.0 mg/kg) plus chloroquine (20.0 mg/kg), in primary treatment, cured infection in 2 of 2 Aotus. No overt toxicity was associated with these treatments. These results indicate that it is feasible to reverse chloroquine-resistance in vivo with a single course of treatment, resulting in infection cure.

SUMMARY

The stated purpose of this contract is to evaluate experimental antimalarial drugs, singly or in combination, against trophozoite-induced infections of Plasmodium falciparum in Aotus lemurinus lemurinus, the Panamanian owl monkey. The Vietnam Smith/RE strain was used and is resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine.

Four derivatives of artemisinin, the active antimalarial principal of the Chinese herb qinghao, were selected for evaluation in the P. falciparum - Aotus model. Two of these derivatives, WR 255131 (arteether) and WR 254986 (artemether) are oil soluble; the water soluble derivatives are WR 255663 (artelinate) and WR 256283 (artesunate). Drugs were administered intramuscularly, q.12h.x3.

Artesunate, at doses of 16.0 to 64.0 mg/kg, cleared parasitemias in 9 of 9 monkeys. At the same range of doses, artelinate cleared parasitemias in 6 of 10 Aotus. Neither drug cured infections, as a recrudescence occurred in all monkeys. These recrudescences were then re-treated with either arteether or artemether.

Both WR 255131 (arteether) and WR 254986 (artemether) were administered at doses ranging from 0.25 to 64.0 mg/kg. Primary treatment with arteether cleared parasitemias in 25/29 monkeys, and cured infection in 15/28 monkeys. Repeat treatment with arteether cured infection in 21 of 30 animals. Overall, arteether cured 36 of 58 (62%) infections. Primary treatment with artemether cleared 24/33 parasitemias and cured 19/33 infections. Repeat treatments with artemether cured 16 of 20 infections. Overall, artemether cured 35 of 49 (71%) infections.

The water soluble, artemisinin derivatives - artesunate and artelinate - effectively cleared parasitemias, but did not cure infections. The oil soluble derivatives - arteether and artemether - cured Vietnam Smith infections, when administered at a total dose equal to or greater than 12.0 mg/kg. The calculated ED_{50} for arteether is 10.0 mg/kg, while the ED_{50} for artemether is 4.0 mg/kg.

Additional experiments were initiated to reverse chloroquine-resistance in vivo by the concomitant administration of chloroquine or quinine and a neuroleptic phenothiazine. All

drugs were administered orally, once daily, for seven days. Quinine (WR 2976), only, at a dose of 40.0 mg/kg, or quinine plus chlorpromazine (WR 2173) at a dose of 20.0 mg/kg suppressed parasitemias of the Vietnam Smith/RE strain of P. falciparum. Retreatments with chlorpromazine (20.0 mg/kg) plus chloroquine (WR 1544), at a dose of 20.0 mg/kg, cured the infection in 2 of 5 Aotus. Primary treatment with chlorpromazine (10.0 mg/kg) plus chloroquine (20.0 mg/kg) cleared parasitemia in each of two monkeys, and cured the infection in one animal. Primary treatment with chlorpromazine (20.0 mg/kg) in combination with chloroquine (20.0 mg/kg) cured the infection in 2 of 2 Aotus. Prochlorperazine (WR 6379) administered at a dose of 10.0 mg/kg with chloroquine (20.0 mg/kg), in a primary treatment, cleared parasitemias, without cure, in 2 of 2 monkeys. Primary treatment with prochlorperazine (20.0 mg/kg) in combination with chloroquine (20.0 mg/kg) cured the infection in 2 of 2 Aotus.

When trials to demonstrate in vivo reversal of chloroquine-resistance were initiated three years ago, the desideratum for demonstration of such reversal was a course of treatment with a drug combination, one of which was chloroquine, during the ascending phase of the primary parasitemia resulting in parasite clearance and infection cure. It is, of course, a given that such drug treatment would not evoke a toxic reaction. Chlorpromazine or prochlorperazine, plus chloroquine, appears to meet these requirements and the combination may be effective in curing human infections of chloroquine-resistant P. falciparum strains.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).

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EXPERIMENTAL PROCEDURES

The monkey-adapted Plasmodium falciparum strain, Vietnam Smith/RE (resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine) was used to induce experimental malaria infections in Aotus lemurinus lemurinus for the evaluation of the antimalarial efficacy of candidate drugs. Infected blood, with sodium citrate (2.5%) as the anticoagulant, from untreated Aotus was diluted appropriately with chilled saline (0.85%), such that each milliliter contained 5,000,000 parasites, and this amount was injected into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm.

Blood films from untreated Aotus, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If a recrudescence occurred, blood films were obtained again on a daily basis.

The schema depicted in Figure 1 represents the designs of a typical drug evaluation study. Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two time per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solutions of water soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8°C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was effected by gastric intubation with a 14 French catheter. The total amount of fluid administered, drug solution or suspension, and rinse was 14 ml.

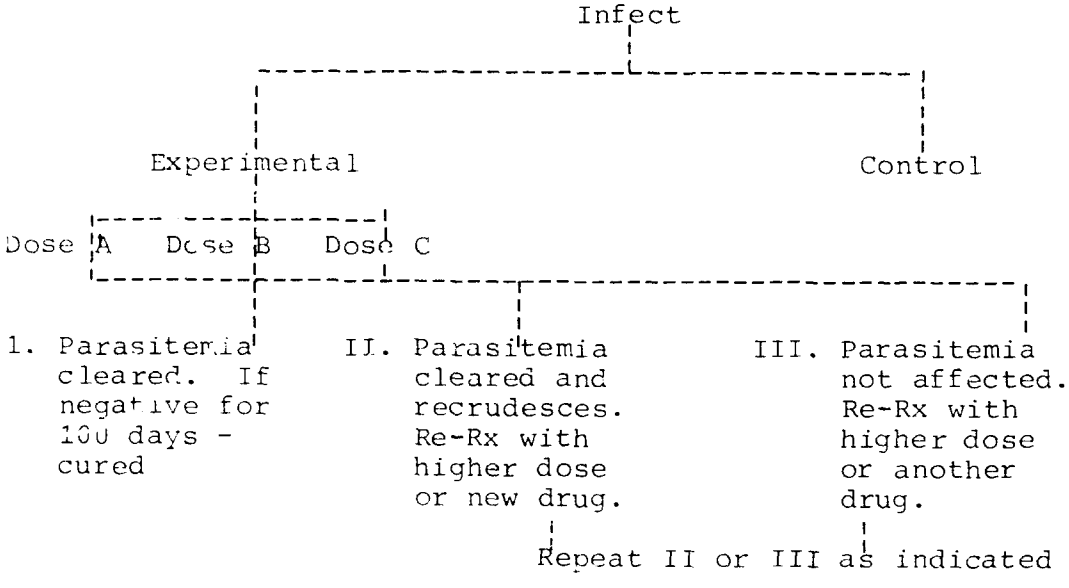
As indicated in the appropriate sections, some water soluble drugs were administered intramuscularly; other water insoluble drugs were diluted in sesame oil and administered intramuscularly.

FIGURE 1

SCHEMA FOR DRUG EVALUATION AGAINST

PLASMODIUM FALCIPARUM

INDUCED INFECTIONS IN AOTUS LEMURINUS LEMURINUS



EVALUATION OF THE ANTIMALARIAL EFFICACY OF FOUR ARTEMISININ DERIVATIVES

A. INTRODUCTION

Artemisinin is the active antimalarial principal of the herb qinghao (Artemisia annua L.) used in China for more than four centuries to combat the chills and fever of malaria. Artemisinin has been identified as a 15-carbon sesquiterpene lactone endoperoxide. Studies in China with patients infected with P. falciparum or P. vivax showed that two derivatives of artemisinin, artemether (oil soluble) and artesunate (water soluble), were significantly active against such infections. Two new artemisinin derivatives were synthesized, arteether (oil soluble) and sodium artelinate (water soluble) and selected for comparison with artemether and artesunate. The antimalarial activity of the four drugs was assessed against infections of the multi-drug resistant Vietnam Smith/RE strain of P. falciparum. All drugs were provided by the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C.

Preliminary data for the antimalarial activity of these four drugs were reported in the previous Annual Report (1988-1989) for this contract.

B. WR 256283AA(BN:BL 28556), artesunate

Pilot evaluation of artesunate at a dose of 64.0 mg/kg im, q.12hx3, indicated that the infection in 1 of 2 Aotus was cured. This frequency and route of administration was used for additional studies of the drug. As shown in Tables 1 and 2, doses of 16.0, 32.0, and 64.0 mg/kg cleared parasitemias in 4 to 6 days after initiating treatment. No infections were cured and the recrudescences were treated with either WR 254986 (artemether) or WR 255131 (arteether). These retreatment data are reported in the appropriate section of this report.

C. WR 255663AH(BN:BL 55866), artelinate

Pilot evaluation of artelinate showed that the intramuscular route of drug administration was better tolerated than the intravenous route. A dose of 64.0 mg/kg (im), q.12hx3, cleared parasitemias, without cure, in each of two Aotus. Results of additional studies are shown in Tables 3 and 4. When administered intramuscularly, q.12hx3, a dose of 16.0 mg/kg cleared parasitemias in 2 of 3 Aotus, a dose of 32.0 mg/kg cleared parasitemias in 2 of 3 monkeys, and parasitemias in 2 of 4 Aotus were cleared with a dose of 64.0 mg/kg. Treatment failures and recrudescences were re-treated with WR 255131 (arteether) OR WR 254986 (artemether), and detailed in the appropriate section.

D. WR 255131AE(BN: BL 48816), arteether

The data in Tables 5, 6, and 9, incorporate results of treatment with this oil soluble artemisinin derivative, arteether, including initial trials reported in the 1988-1989 Annual Report plus re-treatment of recrudescences after administration of artesunate or artelinate. Arteether was administered intramuscularly, q.12hx3. A dose of 0.25 mg/kg suppressed parasitemia in 4 of 4 Aotus. A dose of 1.0 mg/kg cleared parasitemias in 9 of 10 monkeys and cured the infection 1 of 10 animals. All parasitemias were cleared with doses ranging from 4.0 to 64.0 mg/kg. A dose of 4.0 mg/kg cured 12 of 19 infections, 8.0 mg/kg cured 7 of 9 infections, 16.0 mg/kg cured 10 of 10 infections, 32.0 mg/kg cured 2 of 2 infections, and 64.0 mg/kg cured 4/4 of infections.

Overall, parasitemias were cleared in 54 of 59 (91.5%) treated animals, and 36 of 58 (62.1%) infections were cured.

E. WR 254986AB(BN:BL 26767), artemether

All data associated with the antimalarial assessment of artemether, an oil soluble derivative of artemisinin, are shown in Tables 7, 8, and 9. These data include results of the initial evaluation reported in the Annual Report (1988-1989), additional studies initiated during 1989-1990, and re-treatments of recrudescences following administration of artesunate or artelinate. Artemether was administered intramuscularly, q.12h3.

A dose of 0.25 mg/kg suppressed the parasitemia in 4 of 4 Aotus. A dose of 1.0 mg/kg cleared parasitemia in 9 of 10 monkeys. Doses of 4.0, 8.0, 16.0, and 64.0 mg/kg cleared parasitemias in all monkeys. Overall 45 parasitemias were cleared out of a 50 treatments, or 90%. A total of 35 infections out of 49 was cured, or 71.4%.

F. CONCLUSIONS

Both water soluble derivatives of artemisinin, WR 256283 (artesunate) and WR 255663 (artelinate), provoked no toxicity when administered intramuscularly, q.12h, in Aotus. Artesunate, at the three doses administered, cleared parasitemias in 9 of 9 monkeys; however, no infections were cured, as evidenced by recrudescences in all treated animals. Artelinate was less effective than artesunate in clearing parasitemias, as 6 of 10 parasitemias were cleared. These infections were not cured. At the highest dose administered, 64.0 mg/kg, neither artesunate nor artelinate cured infections of the Smith/RE strain.

All doses of the two oil soluble derivatives, WR 255131 (arteether) and WR 254986 (artemether) were well tolerated by Aotus. Artemether was somewhat more effective than arteether in curing Smith/RE infections: primary treatments with artemether cured 19/29 - (66%), while arteether cured 15/28 (54%); retreatments with artemether cured 16/20 (80%), and arteether cured 21/30 (70%). Overall, artemether cured 35 of 49 (71%) of the infections, and arteether cured 36 of 58 (62%) of the infections. Both of the oil soluble derivatives cured Smith/RE infections, while neither of the water soluble derivatives demonstrated curative activity.

TABLE 1

DETAILED ACTIVITY OF WR 256283AA(BL 28556), ARTESUNATE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose* Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Rx		Day Post Treatment						
			1	2	1	2	3	4	5	6	7	8
11409	16.0	18	23	6	0.4	<0.01	<0.01	0	0	0	0	0
11760	16.0	15	13	3	0.2	<0.01	<0.01	0	0	0	0	0
11990	16.0	11	22	3	0.2	<0.01	<0.01	0	0	0	0	0
11763	32.0	10	10	4	0.2	<0.01	0	0	0	0	0	0
11991	32.0	11	13	1	0.1	<0.01	<0.01	0	0	0	0	0
12275	32.0	21	23	5	0.7	<0.01	<0.01	0	0	0	0	0
11018	64.0	12	18	1	0.1	<0.01	<0.01	0	0	0	0	0
11077	64.0	14	22	9	0.5	<0.01	<0.01	0	0	0	0	0
11966	64.0	10	10	1	<0.01	0	0	0	0	0	0	0

* Administered i.m., q.12hx3

TABLE 2

SUMMARY OF THE ACTIVITY OF WR 256283AA(BL 28556), ARTESUNATE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
11409	16.0			+	6	9	Re-Rx, WR 254986
11760	16.0			+	6	10	Re-Rx, WR 255131
11990	16.0			+	6	9	Re-Rx, WR 254986
11763	32.0			+	5	12	Re-Rx, WR 255131
11991	32.0			+	6	12	Re-Rx, WR 254986
12275	32.0			+	6	12	Re-Rx, WR 254986
11018	64.0			+	6	14	Re-Rx, WR 255131
11077	64.0			+	6	14	Re-Rx, WR 255131
11966	64.0			+	4	12	Re-Rx, WR 255131

TABLE 3

DETAILED ACTIVITY OF WR 255663AH(BL 55866), ARTELINATE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose * Mg/Kg	Parasitemia per cmm x 10^3											
		Day Pre-Rx	Day of Rx		Day Post Treatment								
			1	2	1	2	3	4	5	6	7	8	
12306	16.0	3	3	2	0.1	<0.01	0	0	0	0	<0.01	<0.01	a
12347	16.0	1	5	1	<0.01	<0.01	0	0	0	0	<0.01	<0.01	b
12461	16.0	4	13	6	<0.01	<0.01	0	<0.01	<0.01	<0.01	0.8	0.5	b
12301	32.0	1	8	6	0.3	<0.01	0	0	0	0	<0.01	<0.01	b
12313	32.0	2	11	4	0.5	<0.01	0	0	0	0	<0.01	<0.01	a
12460	32.0	5	20	1	<0.01	<0.01	<0.01	0	0	<0.01	0.3	0.8	a
12443	64.0	15	106	100	39	22	9	0.2	<0.01	<0.01	<0.01	0	a
12450	64.0	14	83	43	33	1	0.8	<0.01	<0.01	<0.01	<0.01	0	b
12490	64.0	4	68	34	17	8	2	0.9	<0.01	<0.01	<0.01	0	b
12492	64.0	9	66	87	47	2	1	0.1	<0.01	<0.01	0	<0.01	a

* Administered im, q.12hx3
a Re-Rx, WR 255131, arteether
b Re-Rx, WR 254986, artemether

TABLE 4

SUMMARY OF THE ACTIVITY OF WR 255663AH(BL 5866), ARTELINATE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
12306	16.0			+	5	7	Re-Rx, WR 255131
12347	16.0			+	5	7	Re-Rx, WR 254986
12461	16.0		+		n.a.	n.a.	Re-Rx, WR 254986
12301	32.0			+	5	7	Re-Rx, WR 254986
12313	32.0			+	5	7	Re-Rx, WR 255131
12460	32.0		+		n.a.	n.a.	Re-Rx, WR 255131
12443	64.0		+		n.a.	n.a.	Re-Rx, WR 255131
12450	64.0			+	10	12	Re-Rx, WR 254986
12490	64.0			+	10	11	Re-Rx, WR 254986
12492	64.0		+		n.a.	n.a.	Re-Rx, WR 255131

TABLE 5

DETAILED ACTIVITY OF WR 255131AE(BL 48816), ARTEETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose* Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Rx		Day Post Treatment							
			1	2	1	2	3	4	5	6	7	8
12449	0.25	4	111	37	130	22	2	0.7	2	8	Re-Rx, higher dose	
12472	0.25	1	86	57	18	1	<0.01	<0.01	0.2	Re-Rx, higher dose		
12485	0.25	10	73	197	44	6	11	13	144	Re-Rx, higher dose		
12489	0.25	5	99	78	42	16	122	65	151	Re-Rx, higher dose		
12470	1.0	1	68	65	1	<0.01	<0.01	<0.01	0	0	0	0
12473	1.0	1	70	51	1	<0.01	<0.01	<0.01	0	0	0	0
12487	1.0	10	102	105	5	0.2	<0.01	<0.01	0	0	0	0
12493	1.0	13	72	97	22	1	2	0.2	<0.01	0	0	0
12443r	1.0	5	61	48	1	<0.01	0	0	0	0	0	0
12449r	1.0	8	111	74	1	0.1	0.06	<0.01	0	0	0	0
12460r	1.0	43	715	91	517	345	Re-Rx, higher dose					
12472r	1.0	0.2	2	0.9	0.7	<0.01	<0.01	<0.01	0	0	0	0
12485r	1.0	13	144	65	0.7	0.3	<0.01	0	0	0	0	0
12489r	1.0	65	151	33	1	0.3	<0.01	0	0	0	0	0

* Administered i.m., q.12x3

TABLE 5 (CONT'D.)

DETAILED ACTIVITY OF WR 255131AE(BL 48816), ARTEETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3											
		Day Pre-Rx	Day of Rx		Day Post Treatment								
			1	2	1	2	3	4	5	6	7	8	
12362	4.0	16	34	20	2	0.1	<0.01	<0.01	0	0	0	0	
12363	4.0	13	33	33	0.6	0.06	<0.01	<0.01	0	0	0	0	
12367	4.0	11	34	61	1	0.3	<0.01	<0.01	<0.01	0	0	0	
12410	4.0	11	56	55	2	0.3	<0.01	<0.01	0	0	0	0	
12471	4.0	1	8	6	0.2	<0.01	<0.01	<0.01	0	0	0	0	
12474	4.0	1	117	45	0.9	<0.01	<0.01	<0.01	0	0	0	0	
12486	4.0	10	104	142	18	0.5	<0.01	<0.01	0	0	0	0	
12491	4.0	10	80	71	27	6	2	0.5	<0.01	0	0	0	
12306r	4.0	3	33	5	1	<0.01	<0.01	0	0	0	0	0	
12470r	4.0	79	161	53	1	<0.01	<0.01	<0.01	0	0	0	0	
12487r	4.0	<0.01	0.1	<0.01	0	0	0	0	0	0	0	0	
12492r	4.0	34	444	105	1	<0.01	0	0	0	0	0	0	
12493r	4.0	0.7	1	<0.01	0	0	0	0	0	0	0	0	
12443rr	4.0	<0.01	0.9	<0.01	0	0	0	0	0	0	0	0	
12449rr	4.0	<0.01	<0.01	<0.01	0	0	0	0	0	0	0	0	

TABLE 5 (CONT'D.)

DETAILED ACTIVITY OF WR 255131AF(BL 48816), ARTEETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3											
		Day Pre-Rx	Day of Rx		Day Post Treatment								
			1	2	1	2	3	4	5	6	7	8	
12460rr	4.0	345	203	14	1	0.9	<0.01	0	0	0	0	0	
12472rr	4.0	39	90	8	<0.01	0	0	0	0	0	0	0	
12485rr	4.0	1	17	2	0.2	<0.01	0	0	0	0	0	0	
12489rr	4.0	<0.01	<0.01	0	0	0	0	0	0	0	0	0	
12435	8.0	6	22	3	0.2	<0.01	0	0	0	0	0	0	
12444	8.0	2	5	1	0.1	<0.01	0	0	0	0	0	0	
12456	8.0	14	14	6	0.2	<0.01	0	0	0	0	0	0	
12457	8.0	9	3	0.6	<0.01	<0.01	0	0	0	0	0	0	
11018r	8.0	<0.01	0.1	<0.01	0	0	0	0	0	0	0	0	
11760r	8.0	53	71	234	5	2	0.5	<0.01	0	0	0	0	
12313r	8.0	4	72	5	0.8	<0.01	<0.01	0	0	0	0	0	
12486r	8.0	<0.01	0.2	<0.01	0	0	0	0	0	0	0	0	
12360rr	8.0	0.6	1	1	<0.01	0	0	0	0	0	0	0	

TABLE 5 (CONT'D.)

DETAILED ACTIVITY OF WR 255131AE(BL 48816), ARTEETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Rx		Day Post Treatment							
			1	2	1	2	3	4	5	6	7	8
12359	16.0	17	40	25	5	4	<0.01	<0.01	0	0	<0.01	0
12360	16.0	27	142	42	40	6	0.2	<0.01	<0.01	<0.01	0	<0.01
12412	16.0	9	74	35	2	0.4	<0.01	<0.01	0	0	0	<0.01
12442	16.0	28	40	23	0.2	<0.01	<0.01	<0.01	0	0	0	0
11077r	16.0	<0.01	0.9	<0.01	0	0	0	0	0	0	0	0
11763r	16.0	8	2	71	1	<0.01	0	0	0	0	0	0
11966r	16.0	0.3	1	<0.01	0	0	0	0	0	0	0	0
12474r	16.0	142	126	30	1	<0.01	<0.01	<0.01	<0.01	0	0	0
12493rr	16.0	3	63	4	0.2	<0.01	0	0	0	0	0	0
12460rrr	16.0	0.7	3	<0.01	<0.01	0	0	0	0	0	0	0
12456r	32.0	<0.01	0	0	0	0	0	0	0	0	0	0
12457r	32.0	<0.01	0	0	0	0	0	0	0	0	0	0

TABLE 5 (CONT'D.)

DETAILED ACTIVITY OF WR 255131AF(BL 48816), ARTLETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RF STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3											
		Day Pre-Rx	Day of Rx		Day Post Treatment								
			1	2	1	2	3	4	5	6	7	8	
12354	64.0	18	32	11	1	0.2	<0.01	<0.01	0	0	0	0	
12366	64.0	16	210	10	0.1	0.2	<0.01	<0.01	0	<0.01	0	0	
12400	64.0	14	68	20	0.2	0.09	<0.01	<0.01	0	0	0	0	
12413	64.0	14	105	60	3	0.2	<0.01	<0.01	0	0	0	<0.01	
12424	64.0	14	228	149	80	42	25	7	0.3	<0.01	0	0	

TABLE 6

SUMMARY OF THE ACTIVITY OF WR 255131AE(BL 48816), ARTEETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12449	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12472	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12485	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12489	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12470	1.0			+	7	15	Re-Rx, higher dose
12473	1.0			+	7	n.a.	Cured
12487	1.0			+	7	14	Re-Rx, higher dose
12493	1.0			+	8	11	Re-Rx, higher dose
12443r	1.0			+	5	12	Re-Rx, higher dose
12449r	1.0			+	7	19	Re-Rx, higher dose
12460r	1.0	+			n.a.	n.a.	Re-Rx, higher dose
12472r	1 0			+	7	19	Re-Rx, higher dose
12485r	1.0			+	6	23	Re-Rx, higher dose
12489r	1.0			+	6	25	Re-Rx, higher dose
12362	4.0			+	7	n.a.	Cured
12363	4.0			+	7	n.a.	Cured
12367	4.0			+	12	19	Rx WR 255663
12410	4.0			+	7	18	Rx WR 255663
12471	4.0			+	7	n.a.	Cured
12474	4.0			+	7	14	Re-Rx, higher dose

TABLE 6 (CONT'D.)

SUMMARY OF THE ACTIVITY OF WR 255131AF(BL 48816), ARTEETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12486	4.0			+	7	13	Re-Rx, higher dose
12491	4.0			+	8	n.a.	Cured
12306r	4.0			+	6	21	Re-Rx, higher dose
12470r	4.0			+	7	n.a.	Cured
12487r	4.0			+	3	n.a.	Cured
12492r	4.0			+	5	n.a.	Cured
12493r	4.0			+	3	26	Re-Rx, higher dose
12443rr	4.0			+	3	n.a.	Cured
12449rr	4.0			+	3	n.a.	Cured
12460rr	4.0			+	6	25	Re-Rx, higher dose
12472rr	4.0			+	4	n.a.	Cured
12485rr	4.0			+	5	n.a.	Cured
12489rr	4.0			+	2	n.a.	Cured
12435	8.0			+	5	n.a.	Cured
12444	8.0			+	5	n.a.	Cured
12456	8.0			+	5	21	Re-Rx, higher dose
12457	8.0			+	5	21	Re-Rx, higher dose
11018r	8.0			+	3	n.a.	Cured
11760r	8.0			+	7	n.a.	Cured
12313r	8.0			+	6	n.a.	Cured
12486r	8.0			+	3	n.a.	Cured
12306r	8.0			+	4	n.a.	Cured

TABLE 6 (CONT'D.)

SUMMARY OF THE ACTIVITY OF WR 255131AE(BL 48816), ARTEETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12359	16.0			+	10	n.a.	Cured
12360	16.0			+	11	n.a.	Cured
12412	16.0			+	7	n.a.	Cured
12442	16.0			+	7	n.a.	Cured
11077r	16.0			+	3	n.a.	Cured
11763r	16.0			+	5	n.a.	Cured
11966r	16.0			+	3	n.a.	Cured
12474r	16.0			+	8	n.a.	Cured
12493rr	16.0			+	5	n.a.	Cured
12460rrr	16.0			+	4	n.a.	Cured
12456r	32.0			+	2	n.a.	Cured
12457r	32.0			+	2	n.a.	Cured
12354	64.0			+	7	n.a.	Cured
12366	64.0			+	12	n.a.	Died, Day 51-PostRx*
12400	64.0			+	7	n.a.	Cured
12413	64.0			+	7	n.a.	Cured
12424	64.0			+	9	n.a.	Cured

* Gastrointestinal problem

TABLE 7

DETAILED ACTIVITY OF WR 254986AB(BL 26767), ARTEMETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF *PLASMODIUM FALCIPARUM*

Aotus No.	Dose* Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Rx		Day Post Treatment							
			1	2	1	2	3	4	5	6	7	8
12428	0.25	3	114	67	8	2	0.4	<0.01	<0.01	0.1	Re-Rx, higher dose	
12438	0.25	10	172	117	10	0.3	<0.01	<0.01	<0.01	<0.01	1	Re-Rx
12439	0.25	13	94	154	18	0.6	<0.01	<0.01	<0.01	<0.01	4	Re-Rx
12482	0.25	0.9	96	25	0.4	<0.01	<0.01	<0.01	0	0	<0.01	<0.01
12029	1.0	8	51	40	9	1	0.2	<0.01	<0.01	<0.01	0	0
12421	1.0	5	74	83	23	2	0.7	<0.01	<0.01	<0.01	0	0
12440	1.0	8	76	49	11	0.7	0.1	<0.01	<0.01	<0.01	<0.01	0
12476	1.0	1	56	20	1	<0.01	<0.01	<0.01	0	0	0	0
12428r	1.0	0.1	9	3	0.3	0.2	0.09	<0.01	0	0	0	0
12438r	1.0	1	2	0.1	<0.01	0	0	0	0	0	0	0
12439r	1.0	4	9	5	0.1	<0.01	0	0	0	0	0	0
12461r	1.0	45	507	161	31	1	<0.01	0	0	0	<0.01	<0.01
12482r	1.0	582	117	136	2	0.09	0.1	<0.01	<0.01	0	0	0
12490r	1.01	1	3	0.9	<0.01	0	0	0	0	0	0	0

* Administered i.m.,q.12hx3

TABLE 7 (CONT'D.)

DETAILED ACTIVITY OF WR 254986AB(BL 26767), ARTEMETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Rx		Day Post Treatment							
			1	2	1	2	3	4	5	6	7	8
12390	4.0	15	20	19	0.9	0.06	<0.01	<0.01	0	0	0	<0.01
12398	4.0	35	28	6	0.1	0.04	<0.01	<0.01	0	0	0	0
12414	4.0	13	44	3	0.08	<0.01	<0.01	<0.01	0	0	0	0
12415	4.0	13	22	20	0.1	0.1	0	<0.01	0	0	0	0
12430	4.0	8	140	48	28	1	<0.01	<0.01	<0.01	0	0	0
12453	4.0	2	57	26	8	0.1	<0.01	<0.01	0	0	0	0
12467	4.0	14	130	136	27	31	2	0.2	<0.01	0	0	0
12477	4.0	5	33	22	2	0.1	<0.01	<0.01	0	0	0	0
12029r	4.0	<0.01	<0.01	<0.01	0	0	0	0	0	0	0	0
12347r	4.0	13	48	3	1	<0.01	<0.01	0	0	0	0	0
12421r	4.0	1	2	0.1	<0.01	0	0	0	0	0	0	0
12440r	4.0	0.1	0.4	<0.01	0	0	0	0	0	0	0	0
12450r	4.0	<0.01	0.08	<0.01	0	0	0	0	0	0	0	0
12476r	4.0	14	111	38	4	5	2	<0.01	<0.01	0	0	0
12438rr	4.0	<0.01	<0.01	0	0	0	0	0	0	0	0	0

TABLE 7 (CONT'D.)

DETAILED ACTIVITY OF WR 254986AB(BL 26767), ARTEMETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Rx		Day Post Treatment							
			1	2	1	2	3	4	5	6	7	8
12461rr	4.0	<0.01	<0.01	0	0	0	0	0	0	0	0	0
12482rr	4.0	0.3	0.1	0	0	0	0	0	0	0	0	0
12490rr	4.0	0.2	17	4	0.4	<0.01	<0.01	0	0	0	0	0
11663	8.0	9	42	7	7	0.4	<0.01	<0.01	<0.01	<0.01	<0.01	0
12419	8.0	2	29	6	7	0.6	<0.01	<0.01	<0.01	<0.01	0	0
12459	8.0	13	77	20	20	10	1	<0.01	<0.01	<0.01	0	0
12483	8.0	5	59	18	5	0.1	<0.01	<0.01	0	0	0	0
11409r	8.0	110	57	35	1	0.2	<0.01	0	0	0	0	0
11991r	8.0	0.8	1	<0.01	0	0	0	0	0	0	0	0
12301r	8.0	11	147	262	11	2	<0.01	<0.01	<0.01	0	0	0

TABLE 7 (CONT'D.)

DETAILED ACTIVITY OF WR 254986AB(BL 26767), ARTEMETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Rx		Day Post Treatment							
			1	2	1	2	3	4	5	6	7	8
12166	16.0	20	43	17	0.8	0.2	<0.01	<0.01	0	0	0	0
12388	16.0	18	26	18	0.8	0.1	<0.01	<0.01	0	0	<0.01	0
12403	16.0	23	65	53	0.5	0.2	<0.01	<0.01	0	0	0	0
12401	16.0	16	35	21	0.2	0.1	<0.01	<0.01	0	0	0	<0.01
11990r	16.0	20	5	1	0.2	<0.01	0	0	0	0	0	0
12275r	16.0	1	3	0.9	0.01	0	0	0	0	0	0	0
12393	64.0	21	48	16	0.1	0.2	<0.01	<0.01	0	0	<0.01	<0.01
12399	64.0	29	30	15	0.2	0.07	<0.01	<0.01	<0.01	<0.01	0	<0.01
12402	64.0	39	60	43	0.3	0.04	<0.01	<0.01	0	0	0	<0.01
12404	64.0	30	49	5	5	0.07	0.06	<0.01	<0.01	0	0	0
12425	64.0	11	252	129	49	34	32	5	0.6	<0.01	0	0

TABLE 8

SUMMARY OF THE ACTIVITY OF WR 254986AB(BL 26767), ARTEMETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12428	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12438	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12439	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12482	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12029	1.0			+	9	13	Re-Rx, higher dose
12421	1.0		+		n.a.	n.a.	Re-Rx, higher dose
12440	1.0			+	10	11	Re-Rx, higher dose
12476	1.0			+	7	15	Re-Rx, higher dose
12428r	1.0			+	7	n.a.	Cured
12438r	1.0			+	4	18	Re-Rx, higher dose
12439r	1.0			+	5	n.a.	Cured
12461r	1.0			+	6	7	Re-Rx, higher dose
12482r	1.0			+	8	28	Re-Rx, higher dose
12490r	1.0			+	4	24	Re-Rx, higher dose
12390	4.0			+	7	19	Rx, WR 255663
12398	4.0			+	7	n.a.	Cured
12414	4.0			+	7	n.a.	Cured
12415	4.0			+	7	25	
12430	4.0			+	8	n.a.	Cured
12453	4.0			+	7	n.a.	Cured

* Intercurrent infection

TABLE 8 (CONT'D.)

SUMMARY OF THE ACTIVITY OF WR 254986AB(BL 26767), ARTEMETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12467	4.0			+	8	n.a.	Cured
12477	4.0			+	7	n.a.	Cured
12029r	4.0			+	3	n.a.	Cured
12347r	4.0			+	6	n.a.	Cured
12421r	4.0			+	4	n.a.	Died Day 44 Post-Rx*
12440r	4.0			+	3	n.a.	Cured
12450r	4.0			+	3	n.a.	Cured
12476r	4.0			+	8	n.a.	Cured
12438rr	4.0			+	1	n.a.	Cured
12461rr	4.0			+	1	n.a.	Cured
12482rr	4.0			+	1	n.a	Cured
12490rr	4.0			+	6	n.a.	Cured
11663	8.0			+	10	n.a.	Cured
12419	8.0			+	9	n.a.	Cured
12459	8.0			+	9	n.a.	Cured
12483	8.0			+	7	n.a.	Cured
11490r	8.0			+	6	n.a.	Cured
11991r	8.0			+	3	n.a.	Cured
12301r	8.0			+	8	n.a.	Cured

TABLE 8 (CONT'D.)

SUMMARY OF THE ACTIVITY OF WR 254986AB(BL 26767), ARTEMETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12166	16.0			+	7	n.a.	Cured
12388	16.0			+	12	n.a.	Cured
12403	16.0			+	7	n.a.	Cured
12401	16.0			+	7	n.a.	Cured
11990r	16.0			+	5	n.a.	Cured
12275r	16.0			+	4	n.a.	Cured
12393	64.0			+	11	n.a.	Cured
12399	64.0			+	11	n.a.	Cured
12402	64.0			+	7	n.a.	Cured
12404	64.0			+	8	n.a.	Cured
12425	64.0			+	9	n.a.	Cured

TABLE 9

SUMMARY OF THE ACTIVITY OF FOUR ARTEMISININ DERIVATIVES AGAINST PLASMODIUM FALCIPARUM INFECTIONS

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
SMITH/RE			WR 256283AA(BL 28556), artesunate					
	48.0	16.0	3/3	0/3			3/3	0/3
	96.0	32.0	3/3	0/3			3/3	0/3
	192.0	64.0	3/3	0/3			3/3	0/3
			WR 255663AH(BL 55866), artelinate					
	48.0	16.0	2/3	0/3			2/3	0/3
	96.0	32.0	2/3	0/3			2/3	0/3
	192.0	64.0	2/4	0/4			2/4	0/4
			WR 255131AE(BL 48816), arteether					
	0.75	0.25	0/4	0/4			0/4	0/4
	3.0	1.0	4/4	1/4	5/6	0/6	9/10	1/10
	12.0	4.0	8/8	4/8	11/11	8/11	19/19	12/19
	24.0	8.0	4/4	2/4	5/5	5/5	9/9	7/9
	48.0	16.0	4/4	4/4	6/6	6/6	10/10	10/10
	96.0	32.0			2/2	2/2	2/2	2/2
	192.0	64.0	5/5	4/4			5/5	4/4
			WR 254986AB(BL 26767), artemether					
	0.75	0.25	0/4	0/4			0/4	0/4
	3.0	1.0	3/4	0/4	6/6	2/6	9/10	2/10
	12.0	4.0	8/8	6/8	10/10	9/9	18/18	15/17
	24.0	8.0	4/4	4/4	3/3	3/3	7/7	7/7
	48.0	16.0	4/4	4/4	2/2	2/2	6/6	6/6
	192.0	64.0	5/5	5/5			5/5	5/5

IN VIVO TRIALS TO REVERSE CHLOROQUINE RESISTANCE OF PLASMODIUM FALCIPARUM PARASITES

A. INTRODUCTION

For the past three years, numerous trials have been devoted to the in vivo reversal of chloroquine-resistance of Vietnam Smith/RE parasites. Diverse calcium channel blockers were administered with chloroquine in different regimens. The desideratum to demonstrate in vivo reversal of chloroquine-resistance is drug administration during the primary, ascending phase of the parasitemia, with subsequent parasite clearance and cure of the infection. Such a sequence of events has not been demonstrated heretofore. We did show that desipramine plus chloroquine, administered for three days, will clear parasitemias, but not cure the infection.

Additional in vivo trials to reverse chloroquine-resistance of chloroquine-resistant Smith/RE parasites are reported in subsequent sections.

B. WR 2173AL(BN:BK 20886), chlorpromazine
WR 2976AY(BN:AW 23860), quinine

Chlorpromazine is a psychotropic agent, similar to other drugs previously used for in vivo chloroquine reversal experiments against Vietnam Smith/RE parasites, resistant to maximally tolerated doses of chloroquine, quinine, and pyrimethamine. The data in Tables 10 and 11 show that quinine alone (40.0 mg/kgx7) suppressed the parasitemia in each of two Aotus. *Quinine plus chlorpromazine (20.0 mg/kgx7)* also suppressed the parasitemia in 2 of 2 monkeys. These treatment failures were retreated as indicated in the following section.

C. WR 2173AL(BN: BK 20886), chlorpromazine
WR 1544BM(BN: AR 20613), chloroquine

As shown in Tables 12 and 13, primary treatment with chlorpromazine (20.0 mg/kgx7) plus chloroquine (20.0 mg/kgx7) cleared the parasitemia, with recrudescence, in one Aotus, and suppressed parasitemia in one Aotus. Retreatment of infections, treated initially with chlorpromazine plus quinine, was initiated with chlorpromazine (20.0 mg/kg) plus chloroquine (20.0 mg/kg). One of these monkeys (12494r) became flaccid within 30 minutes after drug administration. Consequently, the dose of chlorpromazine was reduced to 10.0 mg/kg daily. Of these five re-treated monkeys, two died of probable drug toxicity, two were cured of infection, and the parasitemia in one recrudesced.

It must be emphasized that cure of the infection in two Aotus was achieved after two courses of treatment, concomitant with acquired immunity. Similar results have been obtained in previous trials of in vivo reversal of chloroquine resistance. Such cures, however, do not conform to the desideratum of cure following a single treatment regimen, during the ascending phase of the parasitemia.

D. Limited toxicity evaluation of
WR 2173AL(BN:BK 20886), chlorpromazine,
WR 6379AF(BN:BM 1907), prochlorperazine,
each in combination with WR 1544BM(BN:AR 20613), chloroquine.

Although limited in scope, results of the trials detailed in the previous section (C) with chlorpromazine plus chloroquine to reverse chloroquine resistance in vivo were of significant impact to warrant repetition. The two deaths occurring during re-treatment with chlorpromazine plus chloroquine necessitated a limited toxicity evaluation of this drug combination. Also included was prochlorperazine plus chloroquine.

At the doses used (Table 14), the drug combinations were tolerated well, there was no indication of vomiting, diarrhea, or adverse overt responses. There was some anorexia as evidenced by loss of body weight (Table 14). Generally, body weight loss ranged from 5 to 10% of pre-treatment body weight. No animals died during treatment nor any time thereafter.

E. WR 2173AL(BN:BK 20886), chlorpromazine
WR 1544BM(BN:AR 20613), chloroquine

Data in Section D showed that Aotus tolerated a seven day course of treatment with chlorpromazine plus quinine. An experiment was initiated to evaluate further this drug combination against chloroquine-resistant Vietnam Smith/RE parasites. The results are presented in Tables 15 and 16. Chlorpromazine (10.0 and 20.0 mg/kgx7) plus chloroquine (20.0 mg/kgx7) cleared parasitemias within 6 to 7 days after initiation of treatment. A recrudescence occurred in one monkey that had received chlorpromazine (10.0 mg/kgx7) plus chloroquine.

Since there has been no evidence of recrudescences for more than 44 days after primary treatment, it is highly probable that the infections in these animals have been cured. These results appear to fulfill the desideratum of in vivo reversal of chloroquine resistance, viz. one course of treatment during the ascending phase of the primary parasitemia, resulting in clearance of parasitemia and cured of infection.

F. WR 6379AF(BN:BM 1907), prochlorperazine
WR 1544BM(BN:AR 20613), chloroquine

Limited toxicity data (Section D) indicated that prochlorperazine plus chloroquine, administered for 7 days, evoked no overt toxicity in Aotus. This drug combination was used in another trial to reverse chloroquine resistance, in vivo, of Vietnam Smith/RE falciparum parasites. The results of this pilot evaluation are presented in Tables 17 and 18. Prochlorperazine (10.0 mg/kgx7) plus chloroquine (20.0 mg/kgx7) cleared parasitemias in 2 of 2 Aotus; a recrudescence occurred in both animals. Primary treatment with prochlorperazine (20.0 mg/kgx7) plus chloroquine (20.0 mg/kgx7) cleared parasitemias in 2 of 2 Aotus. The absence of recrudescence in these two monkeys for more than 44 days is highly indicative of infection cure. Blood films in the two Aotus (12521r and 12537r) have been parasite negative for more than 16 to 22 days.

G. Limited toxicity evaluation of
WR 267634AC(BN:BM 01916), ketotifen, and
WR 035917AB(BN:BL 08170), cyproheptadine, both
in combination with WR 1544BM(BN:AR 20613), chloroquine

Two tricyclic antihistamines, ketotifen and cyproheptadine were administered to Aotus (cured of malaria infection) to ascertain toxicity, or lack thereof. Each drug, was given orally (once daily) in combination with chloroquine (20.0 mg/kgx7). The body weights of these animals before and after drug treatment are shown in Table 19. On day 1 after the termination of treatment, body weight loss ranged from 1.2% to 6.3%, the highest observed in Aotus 12410 (cyproheptadine, 20.0 mg/kg). By day 14 post-treatment, body weights of 4 of the 6 monkeys were equal to or greater than the pre-treatment body weight. There was no evidence of diarrhea, vomiting, or debilitation during or after treatment.

An experiment is planned to ascertain if ketotifen plus chloroquine, or cyproheptadine plus chloroquine, can reverse chloroquine resistance in vivo.

H. Conclusions

In vivo reversal of chloroquine-resistance is a promising potential in curing P. falciparum infections resistant to this 4-aminoquinoline. The desideratum of such cure is a single course of treatment with chloroquine plus a drug that inhibits the efflux of chloroquine from the parasitized erythrocyte. Results presented in this section for pilot evaluations of chlorpromazine plus chloroquine and prochlorperazine plus chloroquine indicate that both of these neurolopetic phenothiazines plus chloroquine can cure chloroquine-resistant falciparum infections administered at doses tolerated well by Aotus. Each of these drugs is approved for use in humans. If a drug combination proves to be non-toxic, in humans, then chlorpromazine or prochlorperazine, in combination with chloroquine, may be added to the anti-malarial drug armamentarium.

TABLE 10

DETAILED ACTIVITY OF WR 2976AY(AW 23860), QUININE, ALONE AND IN COMBINATION WITH WR 2173AL(BK 20886), CHLORPROMAZINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
12494	40.0a	1	8	55	172	117	228	126	84	79	57	Re-Rx, new drugs
12500	40.0a	1	25	184	80	221	99	389	209	265	296	Re-Rx, new drugs
12498	40.0a 20.0b	1	20	160	161	197	54	49	39	4	1	Re-Rx, new drugs
12508	40.0a 20.0b	2	17	90	82	107	140	81	172	166	350	Re-Rx, new drugs

a WR 2976, quinine
b WR 2173, chlorpromazine

TABLE 11

SUMMARY OF THE ACTIVITY OF WR 2976AY(AW 23860), QUININE, ALONE AND IN COMBINATION WITH WR 2173AL(BK 20886), CHLORPROMAZINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 7 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12494	40.0a		+		n.a.	n.a.	Re-Rx, new drugs
12500	40.0a		+		n.a.	n.a.	Re-Rx, new drugs
12498	40.0a 20.0b		+		n.a.	n.a	Re-Rx, new drugs
12508	40.0a 20.0b		+		n.a.	n.a.	Re-Rx, new drugs

a WR 2976, quinine

b WR 2173, chlorpromazine

TABLE 12

DETAILED ACTIVITY OF WR 2173AL(BK 20886), CHLORPROMAZINE, IN COMBINATION WITH WR 1544BM(AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Treatment						Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
12502	20.0a 20.0b	1	17	97	57	35	19	1	0.9	26	2	Re-Rx
12503	20.0a 20.0b	2	20	234	66	10	1	0.2	<0.01	<0.01	0	0
12494r	20.0a* 20.0b	57	33	39	2	0.8	<0.01	Died, drug toxicity (?)				
12500r	20.0a* 20.0b	296	701	172	34	2	<0.01	<0.01	0	0	0	0
12498r	20.0a* 20.0b	1	1	Died, drug toxicity								
12508r	20.0a* 20.0b	350	493	517	207	58	43	4	0.9	<0.01	0	0
12502r	20.0a* 20.0b	2	59	136	41	30	25	2	0.2	<0.01	0	0

a WR 2173, chlorpromazine

b WR 1544, chloroquine

* Dose of chlorpromazine reduced to 10 mg/kg starting day 2 of Rx

TABLE 13

SUMMARY OF THE ACTIVITY OF WR 2173AL(BK 20886), CHLORPROMAZINE, IN COMBINATION WITH WR 1544BM(AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 7 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Px to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12502	20.0a 20.0b		+		n.a.	n.a.	Re-Rx
12503	20.0a 20.0b			+	9	16	
12494r	20.0a* 20.0b		+		n.a.	n.a.	Died day 6 of Rx, drug toxicity(?)
12500r	20.0a* 20.0b			+	1	n.a.	Cured
12498r	20.0a* 20.0b		n.a.		n.a.	n.a.	Died day 2 of Rx, drug toxicity(?)
12508r	20.0a* 20.0b			+	9	n.a.	Cured
12502r	20.0a* 20.0b			+	9	20	

a WR 2173, chlorpromazine

b WR 1544, chloroquine

* Dose of chlorpromazine reduced to 10.0 mg/kg starting day 2 of Rx

TABLE 14

TOXICITY EVALUATION OF WR 2173AL(BK 20886), CHLORPROMAZINE, AND WR 6379AF(BM 1907), PROCHLORPERAZINE, BOTH IN COMBINATION WITH WR 1544BM(AR 20613), CHLOROQUINE

Monk. No.	Drug mg/kg	Body Weight-gms			
		Pre-Rx	Day Post-Rx		
			1	9	17
12435	10.0a	730	695	674	690
12439	10.0a	819	779	780	790
12444	20.0a	783	725	709	720
12347	20.0a	784	705	792	803
12443	10.0b	884	844	819	789
12419	10.0b	858	813	834	845
12440	20.0b	878	810	823	821
12313	20.0b	880	763	730	718

a. WR 2173AL
b. WR 6379AF
Drugs were administered orally, once daily, for 7 days plus WR 1544BM, 20.0 mg/kg.

TABLE 15

DETAILED ACTIVITY OF WR 2173AL(BK 20886), CHLORPROMAZINE, PLUS WR 1544BM(AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
12510	10.0a 20.0b	12	24	111	43	11	1	<0.01	0	0	0	0
12515	10.0a 20.0b	20	32	142	56	9	0.3	0	0	0	0	0
12516	20.0a 20.0b	18	43	134	14	1	<0.01	0	0	0	0	0
12541	20.0a 20.0b	10	33	81	11	0.8	<0.01	0	0	0	0	0
12510r	20.0a 20.0b	<0.01	0.3	0.6	0.2	0.5	<0.01	0	0	0	0	0

TABLE 16

SUMMARY OF THE ACTIVITY OF WR 2173AL(PK 20886), CHLORPROMAZINE, PLUS WR 1544BM(AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 7 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12510	10.0a 20.0b			+	7	21	Re-Rx, higher dose
12515	10.0a 20.0b			+	6		Negative, > 44 days
12516	20.0a 20.0b			+	6		Negative, > 44 days
12541	20.0a 20.0b			+	6		Negative, > 44 days
12510r	20.0a 20.0b			+	6		Negative, > 12 days

a WR 2173
b WR 1544

TABLE 17

DETAILED ACTIVITY OF WR 6379AF(BM 1907), PROCHLORPERAZINE, PLUS WR 1544BM(AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RF STRAIN OF *PLASMODIUM FALCIPARUM*

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
12521	10.0a 20.0b	14	25	72	15	1	0.1	<0.01	0	0	0	0
12537	10.0a 20.0b	13	51	130	57	1	<0.01	<0.01	0	0	0	0
12539	20.0a 20.0b	17	45	140	35	1	0.2	<0.01	0	0	0	0
12540	20.0a 20.0b	9	35	78	13	0.9	<0.01	0	0	0	0	0
12521r	20.0a 20.0b	2	5	1	1	0.4	<0.01	<0.01	0	0	0	0
12537r	20.0a 20.0b	0.3	11	5	2	1	1	<0.01	0	0	0	0

a WR 6379
b WR 1544

TABLE 18

SUMMARY OF THE ACTIVITY OF WR 6379AF(BM 1907), PROCHLORPERAZINE, PLUS WR 1544BM(AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 7 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12521	10.0a 20.0b			+	7	17	Re-Rx, higher dose
12537	10.0a 20.0b			+	7	13	Re-Rx, higher dose
12539	20.0a 20.0b			+	7		Negative, > 43 days
12540	20.0a 20.0b			+	6		Negative, > 44 days
12521r	20.0a 20.0b			+	7		Negative, > 16 days
12537r	20.0a 20.0b			+	7		Negative, > 22 days

a. WR 6379, prochlorperazine

b. WR 1544, chloroquine

TABLE 19

TOXICITY EVALUATION OF WR 267634AC(BM 01916), KETOTIFEN, AND WR 035917AB(BL 08170), CYPROHEPTADINE, BOTH IN COMBINATION WITH WR 1544BM(AR 20613), CHLOROQUINE

Monkey No.	Drug mg/kg	Body Weight (gms)			
		Days -1	Post Treatment 1	7	14
12352	10.0a	736	717	728	750
12400	20.0a	739	712	723	766
12472	10.0b	786	776	780	780
12384	10.0b	862	823	836	840
12353	20.0b	816	787	788	846
12410	20.0b	799	749	757	776

a WR 035917, oral, once daily x7

b WR 267634, oral, once daily x7

Each monkey also received chloroquine, 20.0 mg/kg, orally, once daily for 7 days.

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